

Sequence identity between human pancreatic cholesterol esterase and bile salt-stimulated milk lipase

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Three overlapping cDNA clones covering the entire primary sequence of the bile salt stimulated lipase in human milk were isolated from a human breast lambda gt10 cDNA library by screening with the rat pancreatic cholesterol esterase cDNA. Nucleotide sequencing of the cDNA showed that the human milk lipase mRNA encodes a 748-residue protein, including a 23-residue signal peptide. The human milk lipase cDNA is highly homologous to rat pancreatic cholesterol esterase, suggesting that the milk lipase may be identical to the cholesterol esterase in human pancreas. This conclusion was confirmed by isolation and sequencing of the cDNA for human pancreatic cholesterol esterase. Analysis of the sequence for the human cholesterol esterase/milk lipase revealed similarities to other serine esterases in three distinct regions of the protein. These domains may represent the active site triads of these proteins.

Cholesterol esterase; Lipase; cDNA cloning; Serine esterase

1. INTRODUCTION

Pancreatic cholesterol esterase catalyzes the hydrolysis of cholesteryl esters to free cholesterol and is important for catalyzing the absorption of dietary cholesterol and fat-soluble vitamins (reviewed in [1]). Recently, Cox et al. [2] have shown that cholesterol esterase expression in the pancreas is low during the juvenile period and increases 1300-fold during maturation. This observation suggests another protein may be involved in mediating cholesterol and vitamin absorption during the developmental period.

The human milk contains a lipase with properties almost identical to the cholesterol esterase of the pancreas [3-6]. Like the pancreatic cholesterol esterase, the catalytic activity of the human milk lipase is activated by the presence of bile salt [3]. The activity of the milk lipase is also inhibited by reagents that inhibit the pancreatic cholesterol esterase [6]. Moreover, antibodies prepared against pancreatic cholesterol esterase cross-reacted with the bile salt stimulated lipase in human milk [7]. Thus, these data suggest that human milk lipase is very similar to pancreatic cholesterol esterase and may be responsible for catalyzing fat absorption during prenatal and neonatal periods.

The cDNA clones and the sequences for rat and

bovine pancreatic cholesterol esterase have been reported recently [8,9]. The N-terminal sequences of both cholesterol esterases are strikingly similar to that of the human milk lipase [5], suggesting that the human milk enzyme may be identical to the cholesterol esterase in mature human pancreas. In the current study, we report the cloning and sequencing of the cDNA for both the human milk lipase and pancreatic cholesterol esterase. The results of this study provided definitive proof of identity between the two proteins.

2. MATERIALS AND METHODS

2.1. Screening of the cDNA library

Full-length rat pancreatic cholesterol esterase cDNA [8] was labeled to high specific activity with [32 P]dATP and used to screen a human breast cDNA library in lambda gt10 vector (Clontech Laboratories, Inc.). According to the manufacturer, the mRNA used for the synthesis of this cDNA library was obtained from human breast tissue excised during the eighth month of pregnancy. The tissue was well-differentiated and was lactational competent. The 32 P-labeled rat cholesterol esterase cDNA was also used to screen two human pancreatic cDNA libraries (Clontech Laboratories, Inc.) to isolate the cDNA for human pancreatic cholesterol esterase. The hybridization and washing conditions for library screening were exactly the same as those described previously [8].

2.2. DNA sequencing

The cDNA from positive clones were isolated by plaque purification. The recombinant DNA was digested with *Eco*RI and then subcloned into pUC-13 plasmid vector for propagation. The resulting plasmids were analyzed by restriction mapping analysis and various restriction fragments of the cDNA were subcloned into M13mp18 or M13mp19 vectors. Single-stranded M13 DNA was prepared and used for sequencing [10] with T7 DNA polymerase. Sequence information was obtained from both strands to cover both orientations and overlapping sequences.

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The nucleotide sequence reported in this paper has been deposited into the GenBank database and has been assigned the accession number M37044

on the amino terminal sequence of the protein [7]. The comparison of the deduced amino acid sequences for the pancreatic cholesterol esterase with milk lipase revealed complete identity between the two proteins.

The human cholesterol esterase/lipase is structurally similar to other serine esterases. As noted previously for the rat pancreatic cholesterol esterase [8], the human enzyme also shares homology with a 63-residue domain in acetylcholinesterase (Table I). This region, encompassing the active site serine of acetylcholinesterase [11], has also been demonstrated recently to contain the active site serine (Ser¹⁹⁴) of rat pancreatic cholesterol esterase [12]. Thus, Ser¹⁹⁴ in human cholesterol esterase/lipase is most likely the active site serine of this protein.

The cholesterol esterase/lipase and cholesterol esterase are similar to acetylcholinesterase and cholinesterase at two additional domains. One of these domains is located at residues 78 to 88 where 100%

identity was observed between the lipases and acetylcholinesterase (Table II). The sequence for cholinesterase [13] is similar with two conserved substitutions (Table II). The other domain with similarities is within residues 430–446. The human lipase and rat pancreatic cholesterol esterase are identical in this region and are 47% similar to the cholinesterases. The similarities increase to 88% if conserved substitutions are considered.

Although the functional significance of these homologous domains remains unknown at the present time, it is noteworthy that the activity of these proteins depends on a catalytic triad involving serine, histidine, and an acidic amino acid [6, 14–15]. The key histidine and the acidic residue have not been identified in any of these proteins. In view of the observation that His⁴³⁵ in cholesterol esterase/lipase is the only histidine conserved with the cholinesterases, this residue may be the key histidine involved in the catalytic activity of the protein.

B	
1143	GGG CTC AGA GGC GGC AAG ACG ACC TTT GAT GTC TAC ACT GAG TOC TGG GCC CAG GAC CCA 1202
356	Gly Leu Arg Gly Ala Lys Thr Thr Phe Asp Val Tyr Thr Glu Ser Trp Ala Gln Asp Pro 375
1203	TCC CAG GAG AAT AAG AAG AAG ACT GTG GTG GAC TTT GAG ACC GAT GTC CTC TTC CTG GTG 1262
376	Ser Gln Glu Asn Lys Lys Lys Thr Val Val Asp Phe Glu Thr Asp Val Leu Phe Leu Val 395
1263	CCC ACC GAG ATT GGC CTA GCC CAG CAC AGA GCC AAT GGC AAG AGT GCC AAG ACC TAC GGC 1322
396	Pro Thr Glu Ile Ala Leu Ala Gln His Arg Ala Asn Ala Lys Ser Ala Lys Thr Tyr Ala 415
1323	TAC CTG TTT TCC CAT CCC TCT CCG ATG CCC GTC TAC CCC AAA TGG GTG GGG GCC GAC CAT 1382
416	Tyr Leu Phe Ser His Pro Ser Arg Met Pro Val Tyr Pro Lys Trp Val Gly Ala Asp His 435
1383	GCA GAT GAC ATT CAG TAC GTT TTC GGG AAG CCC TTC GGC ACC CCC ACG GGC TAC CCG CCC 1442
436	Ala Asp Asp Ile Gln Tyr Val Phe Gly Lys Pro Phe Ala Thr Pro Thr Gly Tyr Arg Pro 455
1443	CAA GAC AGG ACA GTC TCT AAG GCC ATG ATC GCC TAC TGG ACC AAC TTT GCC AAA ACA GGG 1502
456	Gln Asp Arg Thr Val Ser Lys Ala Met Ile Ala Tyr Trp Thr Asn Phe Ala Lys Thr Gly 475
1503	GAC CCC AAC ATG GGC GAC TCG GCT GTG CCC ACA CAC TGG GAA CCC TAC ACT ACG GAA AAC 1562
476	Asp Pro Asn Met Gly Asp Ser Ala Val Pro Thr His Trp Glu Pro Tyr Thr Thr Glu Asn 495
1563	AGC GGC TAC CTG GAG ATC ACC AAG AAG ATG GGC AGC AGC TCC ATG AAG CCG AGC CTG AGA 1622
496	Ser Gly Tyr Leu Glu Ile Thr Lys Lys Met Gly Ser Ser Ser Met Lys Arg Ser Leu Arg 515
1623	ACC AAC TTC CTG CCG TAC TGG ACC CTC ACC TAT CTG CCG CTG CCC ACA GTG ACC GAC CAG 1682
516	Thr Asn Phe Leu Arg Tyr Trp Thr Leu Thr Tyr Leu Ala Leu Pro Thr Val Thr Asp Gln 535
1683	GAG GCC ACC CCT GTG CCC CCC ACA GGG GAC TCC GAG GCC ACT CCC GTG CCC CCC ACG GGT 1742
536	Glu Ala Thr Pro Val Pro Thr Gly Asp Ser Glu Ala Thr Pro Val Pro Pro Thr Gly 555
1743	GAC TCC GAG ACC GCC CCC GTG CCG CCC ACG GGT GAC TCC GGG GGC CCC CCC GTG CCG CCC 1802
556	Asp Ser Glu Thr Ala Pro Val Pro Pro Thr Gly Asp Ser Gly Ala Pro Pro Val Pro Pro 575
1803	ACG GGT GAC TCC GGG GCC CCC CCC GTG CCG CCC ACG GGT GAC TCC GGG GGC CCC CCC GTG 1862
576	Thr Gly Asp Ser Gly Ala Pro Pro Val Pro Pro Thr Gly Asp Ser Gly Ala Pro Pro Val 595
1863	CCG CCC ACG GGT GAC TCC GGG GCC CCC CCC GTG CCG CCC ACG GGT GAC TCC GGG GCC CCC 1922
596	Pro Pro Thr Gly Asp Ser Gly Ala Pro Pro Val Pro Pro Thr Gly Asp Ser Gly Ala Pro 615
1923	CCC GTG CCG CCC ACG GGT GAC TCC GGG GCC CCC CCC GTG CCG CCC ACG GGT GAC TCC GGC 1982
616	Pro Val Pro Pro Thr Gly Asp Ser Gly Ala Pro Pro Val Pro Pro Thr Gly Asp Ser Gly 635
1983	GCC CCC CCC GTG CCG CCC ACG GGT GAC GCG GGG CCC CCC CCC GTG CCG CCC ACG GGT GAC 2042
636	Ala Pro Pro Val Pro Pro Thr Gly Asp Ala Gly Pro Pro Pro Val Pro Pro Thr Gly Asp 655
2043	TCC GGC GCC CCC CCC GTG CCG CCC ACG GGT GAC TCC GGG GGC CCC CCC GTG ACC CCC ACG 2102
656	Ser Gly Ala Pro Pro Val Pro Pro Thr Gly Asp Ser Gly Ala Pro Pro Val Thr Pro Thr 675
2103	GGT GAC TCC GAG ACC GCC CCC GTG CCG CCC ACG GGT GAC TCC GGG GGC CCC CCT GTG CCC 2162
676	Gly Asp Ser Glu Thr Ala Pro Val Pro Pro Thr Gly Asp Ser Gly Ala Pro Pro Val Pro 695
2163	CCC ACG GGT GAC TCT GAG GCT GCC CCT GTG CCC CCC ACA GAT GAC TCC AAG GAA GCT CAG 2222
696	Pro Thr Gly Asp Ser Glu Ala Ala Pro Val Pro Pro Thr Asp Asp Ser Lys Glu Ala Gln 715
2223	ATG CCT GCA GTC AAT AGG TTT TAG CTTCCATGAGCCTTGSTATCAAGAGGCCACAGAGTGGGACCCAC 2293
716	Met Pro Ala Val Ile Arg Phe *
2294	GGGCTCCCTCCCATCTTGAGCTCTTCCTGAATAAAGCCATACCCCTGAAA _n

Table I
Serine active site domains for the esterases

AChe	PGNVGLLDQRMALQVHDNIQFFGGDPKTVTIF <u>GESAGGASVGMHILSPGSRDLFRRAILQSG</u>
	* * * * * * * * * * * * * * * *
HUMAN.CEH	PGNYGLRDQHMAIAWVKRNIAAFGGDPNNITL <u>FGESAGGASVSLQTLSPYNKGLIRRAISQSG</u>
	* * * * * * * * * * * * * * * *
RAT.CEH	PGNFGLRDQHMAIAWVKRNIAAFGGDPDNITIF <u>GESAGAASVSLQTLSPYNKGLIRRAISQSG</u>

Comparison of the active site domains of acetylcholinesterase (AChe) and rat cholesterol esterase (RAT.CEH) with a region of human cholesterol esterase (HUMAN.CEH). The * indicates differences in amino acid. The active site motifs are underlined.

Table II
Putative domain for active site acidic residue

Cholinesterase (90-100)	<u>E</u> <u>D</u> C L Y L N V W I P
ACHe (92-102)	<u>E</u> <u>D</u> C L Y L N I W V P
HUMAN.CEH (78-88)	<u>E</u> <u>D</u> C L Y L N I W V P
RAT.CEH (78-88)	<u>E</u> <u>D</u> C L Y L N I W V P

A conserved sequence between human serum cholinesterase, acetylcholinesterase (ACHe), the human- (HUMAN.CEH), and rat- cholesterol esterase (RAT.CEH). The differences are indicated by * and the putative acidic residue(s) important for enzyme activities are underlined.

Table III
Putative histidine active site domain

ACHe (435-451)	W M G V I <u>H</u> G V E I E F V F G L P
CHOLINESTERASE (433-449)	W M G V M <u>H</u> G Y E I E F V F G L P
	* * * * * # * #
HUMAN.CEH (430-446)	W V G A D <u>H</u> A D D I Q Y V F G K P
RAT.CEH (430-446)	W M G A D <u>H</u> A D D L Q Y V F G K P

Sequence comparison between putative histidine active site domains of acetylcholinesterase (ACHe), human serum cholinesterase, human- (HUMAN.CEH), and rat- cholesterol esterase (RAT.CEH). Conserved substitutions between the serine esterases and the cholesterol esterases are indicated by * while the non-conserved substitutions are indicated by #. The putative active histidines are underlined.

The identity of Glu⁷⁸ and Asp⁷⁹ in the third homologous domain suggests that one or both of these residues may also be important for enzyme activity. Thus, we propose that Glu⁷⁸/Asp⁷⁹, Ser¹⁹⁴, and His⁴³⁵ form the catalytic triad for cholesterol esterase/lipase.

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